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Analysis of beak morphometry of the horned octopus *Eledone cirrhosa*, Lamarck 1798 (Cephalopoda: Octopoda), in the south−eastern Adriatic Sea

Zdravko IKICA[1]*, Vladan VUKOVIĆ[1], Mirko ĐUROVIĆ[1], Aleksandar JOKSIMOVIĆ[1] and Svjetlana KRSTULOVIĆ ŠIFNER[2]

[1] ***Institute of Marine Biology, University of Montenegro, Dobrota b.b., P.O. Box 69, 85330 Kotor, Montenegro***

[2] ***University Department of Marine Studies, University of Split, Livanjska 5/III, 21000 Split, Croatia***

**** Corresponding author, e–mail: zdikica@ac.me***

This paper presents the first information on Eledone cirrhosa *beak morphometry and pigmentation in the Adriatic Sea. The analysis was performed on beaks from 136 female and 82 male specimens caught by demersal trawls in the south–eastern Adriatic. Male–to–female ratio was 0.6:1. Males had larger beaks relative to the mantle length than females, but the difference was not statistically significant. Beak growth was allometrically negative in relation to mantle length and body weight. The situation was similar regarding beak growth in relation to upper and lower mandible hood length, where only the upper rostral length and lower jaw width values in females showed* b*–values higher than 1. Four stages of pigmentation were identified in upper and lower beaks, with a significant degree of overlapping in relation to mantle length.*

Key words: beak, morphometry, horned octopus, *Eledone cirrhosa*, Adriatic Sea

INTRODUCTION

Since cephalopods, when compared to fish or crustaceans, have a relatively greater amount of fleshy tissue directly exposed to digestive processes, their beaks are, due to their chitinous nature, among the few structures that can withstand conditions in predator stomachs (CLARKE, 1962; WOLFF, 1984; LEFKADITOU & BEKAS, 2004). Morphometry, itself a study of variation and change in the size and shape of organisms (PETRIĆ *et al.*, 2010), of cephalopod beaks is of major importance for both the taxonomy of species and for size estimation of individuals from their remains (e.g. from digestive tracts of predators) (LEFKADITOU & BEKAS, 2004).

Early beak research was based on material collected from whales during the industrial whaling era in the first half of the 20th century (XAVIER & CHEREL, 2009), and the most complete guide to cephalopod beaks was published by CLARKE in 1962. Traditionally, morphometrics have been used for species identification and comparison (WOLFF, 1984; LEFKADITOU & BEKAS, 2004; NEIGE, 2006), while recent studies of cephalopod beaks have used morphometric analysis in order to describe geographic variations within a species (BOYLE & NGOILE, 1993;

PIERCE *et al.*, 1994), differences between seasonal spawning groups (KASHIWADA & RECKSIEK, 1978), variation due to growth and sexual dimorphism within a species (BELLO, 2001) and comparison of closely related species (MARTINEZ et al., 2002; PINEDA et al., 2002; PETRIĆ et al., 2010).

Eledone cirrhosa, horned or lesser octopus, is one of the most abundant cephalopods of the Mediterranean Sea, which serves as a prey to several species of demersal fishes, sharks and marine mammals (LEFKADITOU & BEKAS, 2004). The first illustrations of *E. cirrhosa* beaks were given by NAEF (1923) in his monumental work *Die Cephalopoden*, but it was MANGOLD & FIORONI (1966) who presented the detailed description and criteria for species identification, along with mantle length and basic beak dimensions relationships and information regarding pigmentation intensity according to growth from specimens from the Gulf of Lion (LEFKADITOU & BEKAS, 2004). LEFKADITOU & BEKAS (2004) reported the beak morphometry and pigmentation stage analysis of *E. cirrhosa* in the Thracian Sea (Aegean Sea, north–eastern Mediterranean).

Studies of *E. cirrhosa* in the Mediterranean and north–eastern Atlantic have dealt mostly with biology and distribution (BOYLE & KNOBLOCH, 1982; BOYLE, 1983; BOYLE & CHEVIS, 1992; RUNHAM *et al.*, 1997; AGNESI *et al.*, 1998; GRISLEY *et al.*, 1999; SALMAN & KATAĞAN, 1999; BELCARI *et al.*, 2002; ORSI RELINI *et al.*, 2006; DONNALOIA *et al.*, 2010). According to the available data, this paper presents the first analysis of beak morphometry and pigmentation of *E. cirrhosa* in the Adriatic Sea. The aim of the study was to test a method for the reconstruction of individual size through the comparison and correlation of mantle length and body weight and beak characteristics.

MATERIAL AND METHODS

Specimens were obtained monthly, from commercial trawl (20 mm diamond mesh size) catches in Montenegrin territorial and epicontinental waters landed in ports of Herceg Novi, Budva and Bar from April 2009 to June 2012. Specimens for analysis were randomly selected. After taking body measurements (mantle length (ML), to the nearest mm; body weight (BW); with accuracy of 0.01 g), the beaks were removed from specimens and dried for about 24 hours before being stored in paper or nylon bags. Prior to measuring, the beaks were rehydrated in distilled water (KRSTULOVIĆ ŠIFNER, 2004; PETRIĆ *et. al.*, 2010).

Both upper and lower mandible (beak) had the following measurements taken: rostral length (RL), hood length (HL), crest length (CL), wing length (WL), and the distance between jaw angles (JW) (Fig. 1). The amplitude of the lateral wall (ULWa) for the upper beak, and length of base line (LBL) for lower beak were also measured. The terminology used was established by CLARKE (1962) and used by LEFKADI-

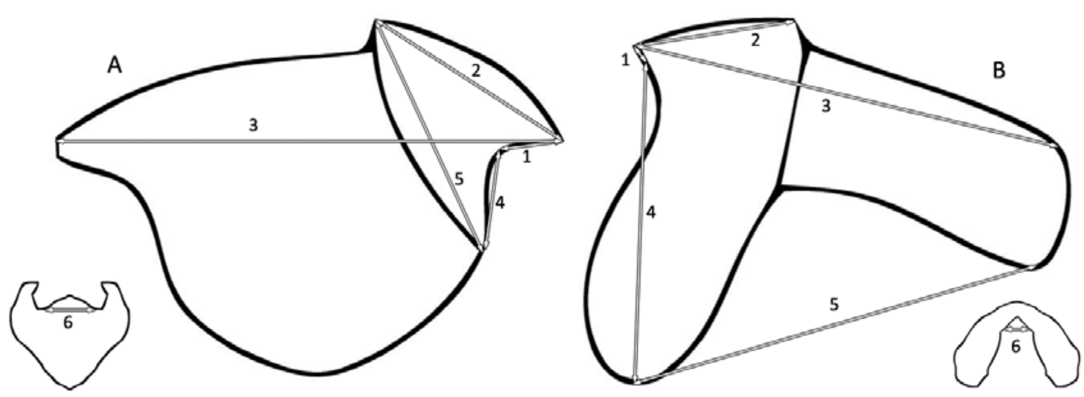

Fig. 1. Beak measurements: (A) upper beak: 1 – rostral length (URL), 2 – hood length (UHL), 3 – crest length (UCL), 4 – wing length (UWL), 5 – lateral wall amplitude (ULWa), 6 – distance between jaw angles (UJW); (B) lower beak: 1 – rostal length (LRL), 2 – hood length (LHL), 3 – crest length (LCL), wing length (LWL), 5 – baseline length, 6 – distance between jaw angles (LJW)

TOU & BEKAS (2004). Measurements were taken with the accuracy of 0.01 mm using a Nikon DC Camera Control Unit DS–L2 with DS–Fi1 camera head, interfaced with the Nikon SMZ800 zoom stereomicroscope.

Analysis of covariance (ANCOVA) was used to determine potential differences in beak measurement growth patterns between sexes, while analysis of variance (ANOVA) was performed on ratios of beak measurements to determine whether there was a statistically significant difference between sexes.

Relationship of different beak measurements to mantle length (ML), body weight (BW) and intermandibular relationships were calculated according to the allometric formula (PEREZ & O'DOR, 2000; PETRIĆ, 2010):

$$y = a \cdot x^{b}.$$

Parameters *a* (intercept) and *b* (slope) were estimated using ordinary least–square regression after transforming the data in common base-10 logarithms:

$$\log y = \log a + b \cdot \log x.$$

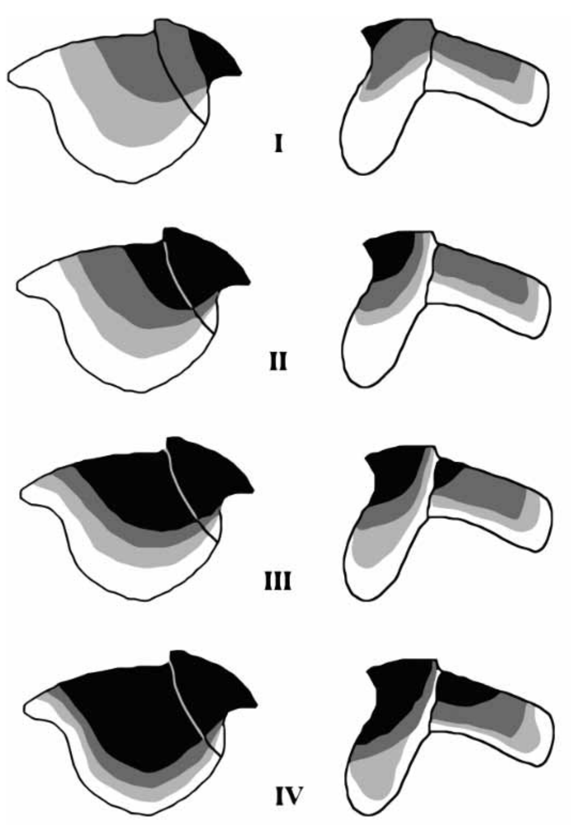

Fig. 2. Pigmentation stages of E. cirrhosa *upper and lower beaks*

The pigmented area of each beak was analysed for possible relation to size increase. Pigmented areas of the amplitude of the lateral wall of the upper beak (UDLWa) and wing length of the lower beak (LDWL) were measured, and expressed in proportion to their respective total lengths (ULWa and LWL).

Four pigmentation stages were identified, according to LEFKADITOU & BEKAS (2004), based on the degree of darkening of the lateral wall of the upper beak and wing in the lower beak. The pigmentation stages were much more easily identified in the lower beak, as shown in Fig. 2.

RESULTS AND DISCUSSION

The beaks of 136 female and 82 male specimens of *E. cirrhosa* were examined. The χ^2–test showed a statistically significant difference between the number of females and males (χ^2 = 13.376, $p < 0.05$).

Analysis of covariance (ANCOVA) showed a statistically significant differences in the lower beak base line growth patterns between sexes (LBL; F = 7.53, $p = 0.007$). The data was therefore processed separately for each sex. Mantle length in sampled male specimens ranged from 50 to 104 mm, with an average of 75.04 ± 10.85 mm ML, while the range of females was from 34 to 127 mm, with an average of 77.42 ± 13.80 mm ML. Body weight of males was in the 47.53 to 295.07 g range (average of 119.22 ± 49.49 g), while females ranged from 12.10 to 420.37 g (average of 130.10 ± 62.19 g).

Upper mandible characteristics and mantle length (ML) ratios

Upper mandible hood length and mantle length ratio (UHL/ML) for the total sample ranged from 2.94% to 7.26%, with a mean value of 5.56 ± 0.64% (Table 1). Minimum and maximum values were 2.94% and 7.26% for females, and 3.23% and 7.23% for males. The average

Table 1. Upper mandible characteristics and mantle length (ML) ratios for females, males and total sample of *E. cirrhosa* in south–eastern Adriatic ($\overline{x}$ – mean value, SE – standard error, SD – standard deviation, CV% – coefficient of variation (%), F – F-statistic)

Ratio	Sex	No	Min	Max	$\overline{x}$	SE	SD	CV%	F
UHL/ML	♀	135	2.94	7.26	5.60	0.06	0.64	11.44	0.77
	♂	81	3.23	7.23	5.51	0.07	0.65	11.76	
	Total	216	2.94	7.26	5.56	0.04	0.64	11.55	
URL/ML	♀	135	0.53	2.57	1.65	0.03	0.38	22.67	0.05
	♂	81	0.82	2.52	1.67	0.04	0.33	19.72	
	Total	216	0.53	2.57	1.66	0.02	0.36	21.56	
UWL/ML	♀	135	2.77	6.05	4.42	0.05	0.57	12.99	2.23
	♂	81	3.28	6.17	4.55	0.07	0.62	13.67	
	Total	216	2.77	6.17	4.47	0.04	0.59	13.29	
ULWa/ML	♀	135	4.93	10.70	8.19	0.08	0.88	10.78	1.50
	♂	81	6.67	10.13	8.33	0.07	0.67	8.07	
	Total	216	4.93	10.70	8.25	0.06	0.81	4.93	
UCL/ML	♀	135	8.86	19.83	15.26	0.13	1.56	10.24	0.08
	♂	81	12.78	19.99	15.32	0.15	1.39	9.06	
	Total	216	8.86	19.99	15.28	0.10	1.50	9.79	
UJW/ML	♀	135	1.29	4.19	3.15	0.04	0.49	15.68	0.01
	♂	81	0.72	4.63	3.15	0.06	0.53	16.92	
	Total	216	0.72	4.63	3.15	0.03	0.51	16.12	

value of UHL/ML relationship for females was 5.60 ± 0.64% for females, and 5.51 ± 0.65 for males. ANOVA showed no statistically significant difference between sexes for the UHL/ML ratio.

Upper mandible rostrum length and mantle length ratio (URL/ML) averaged at 1.66 ± 0.36%, with minimum and maximum at 0.53% and 2.57%, respectively (Table 1). Females had greater range of values for this relationship (0.53% to 2.57%) than males (0.82% to 2.52%), but the mean value was slightly higher for males (1.67 ± 0.33%) than for females (1.65 ± 0.38%). Correlation coefficient was higher for females (22.67%), corresponding to the greater range of values, than in males (19.72%). Correlation coefficient for the total sample was 21.56%. Analysis of variance did not show statistically significant difference between sexes for the URL/ML ratio.

Upper mandible wing length and mantle length ratio (UWL/ML) was in the 2.77% to 6.05% range, with an average value of 4.47 ± 0.59% (Table 1). Values for females were found in the 2.77% to 6.05% range, with a mean of 4.42 ± 0.57%, while males had an average of 4.55 ± 0.62% and a range from 3.28% to 6.17%. ANOVA did not show statistically significant difference between values of UWL/ML ratio for females and males.

The mean value of amplitude of the upper mandible lateral wall and mantle length ratio (ULWa/ML) for the entire sample was estimated at 8.25 ± 0.81%, with range from 4.93% to 10.70%. This corresponds to the ULWa/ML range for females (Table 1), while the range of this ratio was narrower for males (6.67% to 10.13%). The mean value of the ULWa/ML ratio for females was 8.19 ± 0.88%, and 8.33 ± 0.67% for males. No statistically significant difference between sexes was found (ANOVA).

The values of the upper mandible crest length and mantle length ratio (UCL/ML) ranged from 8.86% to 19.83% for females, 12.78% to 19.99% for males, and from 8.86% to 19.99% for total sample (Table 1). Mean values were similar for all three groups, 15.26 ± 1.56% for females, 15.32 ± 1.39% for males and 15.28 ± 1.50% for total sample. No statistically significant difference between sexes was found according to the analysis of variance.

Males had somewhat wider range of values of the upper mandible distance between jaw angles and mantle length (UJW/ML) (0.72% to 4.63%) compared to females (1.29% to 4.19%), and the range of the total sample corresponded

Table 2. Lower mandible characteristics and mantle length (ML) ratios for females, males and total sample of *E. cirrhosa* in south–eastern Adriatic ($\bar{x}$ – mean value, SE – standard error, SD – standard deviation, CV% – coefficient of variation (%), F – F-statistic)

Ratio	Sex	No	Min	Max	$\bar{x}$	SE	SD	CV%	F
LHL/ML	♀	136	2.46	8.29	4.24	0.06	0.67	15.76	0.37
	♂	81	2.84	8.26	4.30	0.08	0.73	16.86	
	Total	217	2.46	8.29	4.26	0.05	0.69	16.16	
LRL/ML	♀	136	0.41	1.67	0.92	0.02	0.22	24.12	2.21
	♂	81	0.36	1.87	0.97	0.03	0.27	27.40	
	Total	217	0.36	1.87	0.94	0.02	0.24	25.55	
LWL/ML	♀	136	5.63	11.27	8.96	0.08	0.97	10.80	0.00
	♂	81	7.59	11.67	8.96	0.09	0.80	8.95	
	Total	217	5.63	11.67	8.96	0.06	0.90	10.12	
LCL/ML	♀	136	5.85	13.73	9.84	0.10	1.16	11.75	0.28
	♂	80	8.09	12.71	9.92	0.10	0.93	9.34	
	Total	216	5.85	13.73	9.87	0.07	1.08	10.89	
LBL/ML	♀	136	6.81	15.13	11.55	0.12	1.36	11.75	4.84*
	♂	80	9.46	14.22	11.17	0.11	0.94	8.38	
	Total	216	6.81	15.13	11.41	0.08	1.23	10.78	
LJW/ML	♀	136	0.99	2.73	1.97	0.03	0.34	17.38	1.99
	♂	81	1.32	3.33	2.04	0.04	0.38	18.43	
	Total	217	0.99	3.33	1.99	0.02	0.36	17.83	

* Statistically significant ($p < 0.05$)

to that of males (Table 1). The mean value of this relationship was identical for all three groups, 3.15%, with the differences in standard deviations ($SD_♀$ = 0.49%, $SD_♂$ = 0.53%, SD_{TOT} = 0.51%). ANOVA did not show a statistically significant difference between sexes.

With the exception of UHL/ML and UJW/ML ratios, all other ratios had shown higher mean values for males (UHL/ML had higher values for females, and in UJW/ML the values were identical), indicating that in general, males have larger beaks in proportion to mantle length.

Lower mandible characteristic and mantle length (ML) ratios

Lower mandible hood length and mantle length ratio (LHL/ML) for the total sample ranged from 2.46% to 8.29%, with a mean value of 4.26 ± 0.69% (Table 2). For males, the range was from 2.84% to 8.26%, and from 2.46% to 8.29% for females. The mean values for males and females were 4.30 ± 0.73% and 4.24 ± 0.67, respectively. Analysis of variance showed no statistically significant difference between sexes.

Lower mandible rostrum length and mantle length ratio (LRL/ML) had the mean length estimated at 0.94 ± 0.24%, with a range from 0.36% to 1.87% (Table 2). For males, the range was identical to that of the total sample, while the mean value was 0.97 ± 0.27%. For females, the LRL/ML ratio ranged from 0.41% to 1.67%, with a mean value 0.92 ± 0.22%. ANOVA did not show a statistically significant difference between the sexes.

Lower mandible wing length and mantle length ratio (LWL/ML) ranged from 5.63% to 11.67%. It ranged from 5.63% to 11.27% for females and from 7.59% to 11.67% for males (Table 2). The average values were identical for females, males and the total sample (8.96%), while standard deviations were 0.80% for males, 0.90% for the total sample and 0.97% for females. No statistically significant difference was found between females and males.

The range of the lower mandible crest length and mantle length ratio (LCL/ML) for females (5.85% to 13.37%) was greater than that of males (8.09% to 12.71%), and corresponded to the range of the total sample (Table 2). The mean value was higher for males (9.92 ± 0.93%)

than for females (9.84 ± 1.16%) and total sample (9.87 ± 1.08%). Analysis of variance showed no statistically significant difference between sexes.

Length of the base line and mantle length (LBL/ML) ratio of the lower mandible had the greatest range (6.81% to 15.11%), highest mean value (11.55 ± 1.36%) and coefficient of variation (11.75%) for females (Table 2). Somewhat lower values were recorded for the total sample ($\bar{x}$ ± SD = 11.41 ± 1.23%, CV = 10.78%), while the range was identical to that of females. In males it ranged from 9.46% to 14.22%, with a mean value of 11.17 ± 0.94%, and the coefficient of variation of 8.38%. ANOVA showed that there was a statistically significant difference between females and males for the LBL/ML ratio.

Distance between jaw angles and mantle length ratio (LJW/ML) of the lower mandible for the total sample ranged from 0.99% to 3.33%, with a mean value of 1.99 ± 0.36% (Table 2). The range was from 0.99% to 2.73% for females and from 1.32% to 3.33% for males, with mean values of 1.97 ± 0.34% for females and 2.04 ± 0.38% for males. There were no statistically significant differences between sexes for the LJW/ML.

Similar to the results obtained for the upper mandible (beak), the mean values of the lower mandible ratios were generally higher in males than in females, with the exception of LBL/ML ratio where females had higher values and LWL/ML where the values were identical in both sexes. These results also indicated larger beak size in males relative to mantle length. It is interesting to note that *E. moschata*, horned octopus' sister species, had the values reversed – generally, the beaks are larger in females relative to mantle length (MANGOLD & FIORONI, 1966; KRSTULOVIĆ ŠIFNER, 2004).

Beak measurements in relation to mantle length and body weight

Beak growth in relation to mantle length (ML) showed negative allometry, with values of the slope parameter b consistently lower than 1 (where b = 1 indicates isometric growth) (Table 3). In females, upper beak hood length (UHL) and the amplitude of the lateral wall (ULWa) relationship to mantle length (ML) had slope values (b) relatively close to 1 (b_{UHL} = 0.901, b_{ULWA} = 0.923). In males, upper beak crest length (UCL) and lower beak base length (LBL) had slope values (b) estimated at above 0.9 (b_{UCL} = 0.906, b_{LBL} = 0.936).

The lowest correlation coefficients were found for rostral lengths, both in upper (URL) and lower jaw (LRL) in relation to mantle length (ML), for both females and males (Table 3). This has been somewhat expected, as the rostral length cannot be accurately determined in octopod species (CLARKE, 1962; LEFKADITOU & BEKAS, 2004), and the exoskeleton of crustaceans, which are the preferred prey of eledonid species (BOYLE, 1983; ŞEN, 2007; ŞEN & AKYOL, 2011), probably contribute to the erosion of the beak rostrum.

Relationship of upper and lower beak hood lengths (UHL, LHL) and crest lengths (UCL, LCL) to body weight (BW) also showed negative allometry (b < 3) for both sexes (Table 3). The slope parameter b for hood length to body weight relationship (HL-BW) was much higher in females ($b_{♀UHL}$ = 2.741, $b_{♀LHL}$ = 2.067) than in males ($b_{♂UHL}$ = 1.790, $b_{♂LHL}$ = 1.533). The differences in values for crest length to body weight relationship (CL−BW), while still higher in females, were not as prominent. The correlation coefficients were higher for CL−BW for both sexes than for HL−BW relationship, and higher in females than in males (Table 3).

Since LEFKADITOU & BEKAS (2004) did not find a statistically significant difference between female and male beaks, they did the linear regression analysis on the total sample (both sexes pooled together). Compared to the results from that study, females from the south–east Adriatic (this study) generally had higher values of relationship between beak characteristics and mantle length. The exceptions were rostral length and distance between jaw angles for both upper and lower mandible (URL−ML, LRL−ML, UJW−ML, LJW−ML). Males showed mostly lower growth rate for the upper beak (with the exception of UCL−ML and ULWa−

Table 3. Relationship between lower and upper beak measurements and mantle length (ML) and body weight (BW), separately for females and males of *E. cirrhosa* in Montenegrin waters (*a* –intercept, *b* – slope, *r* – correlation coefficient, F – F-statistic, *p* – significance of each model)

X	Y	Sex	Number of individuals	*a*	*b*	*r*	F	*p*
UHL	ML	♀	135	20.749	0.901	0.783	210.79	< 0.001
		♂	81	29.923	0.648	0.593	42.97	< 0.001
URL		♀	135	71.121	0.330	0.509	46.53	< 0.001
		♂	81	69.823	0.315	0.525	30.07	< 0.001
UWL		♀	135	30.200	0.766	0.761	183.18	< 0.001
		♂	81	37.931	0.555	0.628	51.34	< 0.001
ULWa		♀	135	14.125	0.923	0.819	271.27	< 0.001
		♂	81	15.205	0.872	0.839	187.51	< 0.001
UCL		♀	135	8.521	0.895	0.838	314.75	< 0.001
		♂	81	8.236	0.906	0.793	134.03	< 0.001
UJW		♀	135	47.098	0.559	0.639	91.49	< 0.001
		♂	81	60.256	0.252	0.410	15.96	< 0.001
LHL		♀	136	33.574	0.706	0.675	112.45	< 0.001
		♂	81	37.239	0.606	0.592	43.03	< 0.001
LRL		♀	136	87.297	0.354	0.494	43.14	< 0.001
		♂	81	78.705	0.158	0.329	9.53	0.003
LWL		♀	136	14.555	0.865	0.817	269.06	< 0.001
		♂	81	16.106	0.808	0.816	157.53	< 0.001
LCL		♀	136	12.794	0.888	0.782	211.44	< 0.001
		♂	80	13.335	0.861	0.780	121.38	< 0.001
LBL		♀	136	14.655	0.760	0.803	243.80	< 0.001
		♂	80	10.280	0.936	0.826	168.24	< 0.001
LJW		♀	136	62.230	0.520	0.744	165.98	< 0.001
		♂	81	62.951	0.412	0.683	68.90	< 0.001
UHL	BW	♀	135	2.192	2.741	0.817	266.70	< 0.001
		♂	81	8.956	1.790	0.599	44.17	< 0.001
UCL		♀	135	0.162	2.680	0.861	380.18	< 0.001
		♂	81	0.268	2.479	0.793	133.71	< 0.001
LHL		♀	136	10.375	2.067	0.677	113.09	< 0.001
		♂	80	19.231	1.533	0.547	33.34	< 0.001
LCL		♀	136	0.547	2.663	0.802	241.25	< 0.001
		♂	80	1.034	2.343	0.775	117.52	< 0.001

ML) and higher for the lower beak (exceptions were LRL−ML and LJW−ML) compared to the results from the Thracian Sea. Comparison of hood and crest length in relation to body weight showed higher growth rate for females from the south–east Adriatic in all cases compared to the results of LEFKADITOU & BEKAS (2004). Adriatic males, however, showed higher growth rate only for crest length and body weight relationship (UHL−ML, LHL−ML), but the hood length to body weight relationships (UHL−ML, LHL−ML) were lower than those from the Thracian Sea.

Intermandibular relationships

Ratios of measured beak characteristics to hood length (HL) of the corresponding mandible showed statistically significant differences between sexes in the UWL/UHL ($F = 4.65$, $p < 0.05$), ULWa/UHL ($F = 6.53$, $p < 0.05$) and LBL/UHL ($F = 4.85$, $p = 0.05$) ratios (Table 4). Coefficients of variation were generally lower than 25% (the only exception was LRL/LHL), implying a homogeneity of the sample (Table 4).

Linear regression analysis of beak measurements in relation to the upper and lower beak hood length (UHL, LHL) showed positive allometry ($b > 1$) for upper jaw rostrum length

Table 4. Ratios of upper and lower mandible characteristics (%) and hood length (HL) for females, males and total sample of *E. cirrhosa* ($\bar{x}$ – mean value, SE – standard error, SD – standard deviation, CV% – coefficient of variation (%), F – F-statistic)

Ratio	Sex	No	Min	Max	$\bar{x}$	SE	SD	CV%	F
URL/UHL	♀	135	10.20	43.64	29.65	0.53	6.14	20.70	0.86
	♂	81	18.06	41.92	30.44	0.67	6.02	19.77	
	Total	216	10.20	43.64	29.94	0.41	6.09	20.34	
UWL/UHL	♀	135	56.12	123.59	79.69	0.93	10.82	13.58	4.65*
	♂	81	57.17	168.73	83.72	1.85	16.61	19.84	
	Total	216	56.12	168.73	81.20	0.91	13.40	16.50	
ULWa/UHL	♀	135	107.65	194.29	147.27	1.17	13.60	9.23	6.53*
	♂	81	113.94	219.92	152.78	1.99	17.88	11.70	
	Total	216	107.65	219.92	149.33	1.06	15.54	10.40	
UCL/UHL	♀	135	199.63	361.53	274.46	2.26	26.23	9.56	2.24
	♂	81	225.17	419.19	280.49	3.61	32.45	11.57	
	Total	216	199.63	419.19	276.92	1.96	28.79	10.41	
UJW/UHL	♀	135	26.13	75.44	56.48	0.69	8.05	14.25	0.87
	♂	81	12.27	91.11	57.67	1.17	10.57	18.33	
	Total	216	12.27	91.11	56.93	0.62	9.07	15.93	
LRL/LHL	♀	136	9.77	39.94	21.96	0.47	5.51	25.09	1.37
	♂	81	7.55	40.97	22.95	0.75	6.74	29.39	
	Total	217	7.55	40.97	22.33	0.41	6.00	26.89	
LWL/LHL	♀	136	101.99	312.78	214.06	2.35	27.44	12.82	0.10
	♂	81	101.14	299.96	212.73	3.63	32.65	15.35	
	Total	217	101.14	312.78	213.56	2.00	29.43	13.78	
LCL/LHL	♀	136	106.85	327.66	234.89	2.52	29.37	12.50	0.01
	♂	80	101.56	317.00	234.43	3.77	33.75	14.40	
	Total	216	101.56	327.66	234.72	2.11	30.99	13.20	
LBL/LHL	♀	136	120.40	389.28	276.42	3.41	39.79	14.40	4.85*
	♂	80	113.40	376.61	264.27	4.25	38.04	13.39	
	Total	216	113.40	389.28	271.92	2.69	39.50	14.53	
LJW/LHL	♀	136	25.26	72.17	47.13	0.80	9.30	19.73	1.06
	♂	81	15.47	93.30	48.61	1.29	11.62	23.91	
	Total	217	15.47	93.30	47.68	0.69	10.23	21.45	

* Statistically significant (p = 0.05)

(URL) and lower jaw angle width (LJW) for females (Table 5). This is different from the analysis by LEFKADITOU & BEKAS (2004), which showed positive allometric growth for all upper beak measurements, as well as for the lower beak wing length (LWL). Reported correlation coefficients (r) were also higher in the Thracian Sea, ranging from 0.515 to 0.935, whereas the r values in south-eastern Adriatic ranged from 0.276 to 0.85 (Table 5).

The highest correlation coefficient was determined for the upper beak lateral wall amplitude and hood length relationship (ULWa−UHL, r = 0.85), while the lowest was for the lower beak rostrum length and hood length relationship (LRL−LHL, r = 0.276) (Table 5).

Beak pigmentation analysis

Beaks of *E. cirrhosa* were categorised according to four stages of pigmentation (Fig. 2) (LEFKADITOU & BEKAS, 2004). Analysis of pigmentation stages of upper and lower mandibles showed a significant degree of overlapping between the stages (Tables 6 and 7).

Analysis of variance (ANOVA) did not show a statistically significant difference between sexes in any of the pigmentation stages for UDLW/ULWa (Table 6). Mean values were similar between males and females, with the greatest difference found in Stage 1 (1.30%), and the least in Stage 4 (0.15%). Coefficients of variation were similar between sexes, except in Stage

Table 5. Intercept (*a*) and slope (*b*) parameters of upper and lower beak measurements expressed as a function of hood length (HL) for *E. cirrhosa* in Montenegrin waters (*r* – correlation coefficient, F – F-statistic, *p* – significance of each model)

X	Y	Sex	No	*a*	*b*	*r*	F	*p*
UHL	URL	♀	135	0.266	1.059	0.597	73.54	< 0.001
		♂	81	0.313	0.967	0.531	30.98	< 0.001
	UWL	♀	135	1.017	0.824	0.721	144.05	< 0.001
		♂	76	1.369	0.622	0.575	36.04	< 0.001
	ULWa	♀	135	1.776	0.867	0.850	345.45	< 0.001
		♂	81	2.347	0.689	0.656	59.59	< 0.001
	UCL	♀	135	3.111	0.910	0.844	329.95	< 0.001
		♂	81	4.757	0.619	0.647	57.02	< 0.001
	UJW	♀	135	0.642	0.903	0.687	118.81	< 0.001
		♂	80	0.779	0.783	0.563	36.19	< 0.001
LHL	LRL	♀	135	0.318	0.657	0.429	30.08	< 0.001
		♂	80	0.360	0.578	0.276	6.38	< 0.05
	LWL	♀	135	2.671	0.806	0.777	202.45	< 0.001
		♂	80	3.569	0.543	0.525	29.80	< 0.001
	LCL	♀	135	3.133	0.749	0.775	200.24	< 0.001
		♂	79	3.998	0.532	0.575	38.03	< 0.001
	LBL	♀	135	3.214	0.866	0.747	167.83	< 0.001
		♂	79	4.584	0.517	0.574	37.87	< 0.001
	LJW	♀	134	0.445	1.032	0.668	106.23	< 0.001
		♂	78	0.673	0.687	0.462	2054	< 0.001

Table 6. Proportion of darkened part of lateral wall amplitude (UDLWa) in upper beak of *E. cirrhosa* females and males in Montenegrin waters ($\bar{x}$ – mean value, SE – standard error, SD – standard deviation, CV% – coefficient of variation (%), F – F-statistic)

Pigmentation stage	Sex	No	Min	Max	$\bar{x}$	SE	SD	CV%	F
1	♀	6	61.15	76.36	66.55	2.20	5.40	8.11	0.11
	♂	8	60.41	87.40	67.85	2.94	8.32	12.26	
2	♀	16	70.03	87.06	79.21	1.19	4.75	6.00	0.02
	♂	13	70.47	92.42	78.88	1.81	6.51	8.26	
3	♀	62	74.90	92.94	82.99	0.56	4.44	5.35	1.55
	♂	39	73.60	97.56	84.21	0.86	5.36	6.37	
4	♀	47	62.81	98.10	88.87	0.89	6.10	6.87	0.01
	♂	19	75.00	98.65	88.72	1.80	7.83	8.82	

1 of the upper mandible, where CV of males was about 50% higher than that of females (Table 6).

Similar to the upper mandible, no statistically significant differences were noticed in the LDLW/LWL ratio (Table 7), and there was no noticeable difference between mean values of the ratio between sexes. Coefficients of variation had low values (lower than 7%) for all pigmentation stages except for Stage 1 where they have been noticeably higher (17.34% for females and 21.86 for males) (Table 7).

Linear regression analysis of the relationship of darkened area of the upper mandible lateral wall and hood length (UDLWa−UHL) and crest length (UDLWa−UCL) in females was allometrically negative ($b < 1$) (Table 8). Correlation coefficient was somewhat higher in UDLWa−UCL ($r = 0.868$) than in the UDLWa−UHL relationship ($r = 0.844$). Analysis showed much lower parameters in males, both in slope ($b_{\mathrm{UDLWa-UCL}} = 0.532$, $b_{\mathrm{UDLWa-UHL}} = 0.485$) and in correlation coefficient ($r_{\mathrm{UDLWa-UCL}} = 0.701$,

Table 7. Proportion of darkened part of wing length (LWL) in lower beak of *E. cirrhosa* females and males in Montenegrin waters ($\bar{x}$ – mean value, SE – standard error, SD – standard deviation, CV% – coefficient of variation (%), F – F-statistic)

Pigmentation stage	Sex	No	Min	Max	$\bar{x}$	SE	SD	CV%	F
1	♀	12	37.02	65.37	56.87	2.85	9.86	17.34	0.00
	♂	2	48.33	66.00	57.16	8.84	12.50	21.86	
2	♀	24	63.50	75.56	69.95	0.69	3.39	4.84	0.79
	♂	15	66.42	76.34	70.86	0.69	2.69	3.79	
3	♀	26	72.47	83.60	77.74	0.49	2.48	3.19	3.60
	♂	8	73.50	78.91	75.95	0.73	5.15	2.25	
4	♀	63	71.24	99.45	87.03	0.70	5.52	6.34	0.01
	♂	49	78.11	99.49	86.95	0.73	5.15	5.92	

Table 8. Relationship of upper mandible pigmented area of the lateral wall (UDLWa) to hood length (UHL), crest length (UCL) and mantle length (ML) and relationship of the pigmented area of lower mandible wing (LDLW) to hood length (LHL, crest length (LCL) and mantle length (ML), for females and males of *E. cirrhosa* in Montenegrin waters (*a* – intercept*, b* – slope, *r* – correlation coefficient, F – F-statistic, *p* – significance of each model)

Y	X	Sex	No	*a*	*b*	*r*	F	*p*
UDLWa	UHL	♀	134	1.303	0.714	0.844	332.00	< 0.001
		♂	81	1.842	0.485	0.612	47.29	< 0.001
	UCL	♀	134	3.142	0.789	0.868	403.57	< 0.001
		♂	81	4.762	0.532	0.701	76.43	< 0.001
	ML	♀	136	20.989	0.782	0.811	257.90	< 0.001
		♂	81	27.164	0.617	0.712	81.33	< 0.001
LDLW	LHL	♀	130	1.508	0.442	0.749	163.88	< 0.001
		♂	79	1.633	0.383	0.554	34.12	< 0.001
	LCL	♀	131	3.385	0.469	0.815	256.48	< 0.001
		♂	80	3.282	0.472	0.734	91.38	< 0.001
	ML	♀	133	32.659	0.503	0.766	185.58	< 0.001
		♂	80	28.708	0.557	0.786	126.00	< 0.001

$r_{UDLWa-UHL}$ = 0.612). Regression analysis of the UDLWa−ML relationship also showed higher values for females ($b_{♀}$ = 0.782; $b_{♂}$ = 0.617; $r_{♀}$ = 0.811; $r_{♂}$ = 0.712).

Linear regression analysis for LDLW−LHL relationship showed lower values of the slope (*b*) and correlation coefficient (*r*) for males ($b_{♂}$ = 0.383; $r_{♂}$ = 0.554) than for females ($b_{♀}$ = 0.442; $r_{♀}$ = 0.749), while the LDLW−LCL relationship had higher values of *b* for males (Table 8). Males had correlation coefficient higher than females only for LDLW−ML relationship (Table 8).

Both sexes showed a significant overlap of the pigmentation stages as function of mantle length (ML) (Fig. 3). Pigmentation stages for both upper and lower mandible and both sexes appear at similar mantle lengths. In comparison, the relationship between pigmentation stages and mantle lengths appears to be much more defined in the research from the Thracian Sea, and shows less overlap between pigmentation stages and mantle lengths (LEFKADITOU & BEKAS, 2004).

Compared to the results reported in the Thracian Sea, the slope parameter (*b*) value of the linear regression of the UDLWa−UHL, LDWL−LHL, UDLWa−ML and LDWL−ML relationships is lower in the south–eastern Adriatic (LEFKADITOU & BEKAS, 2004), indicating

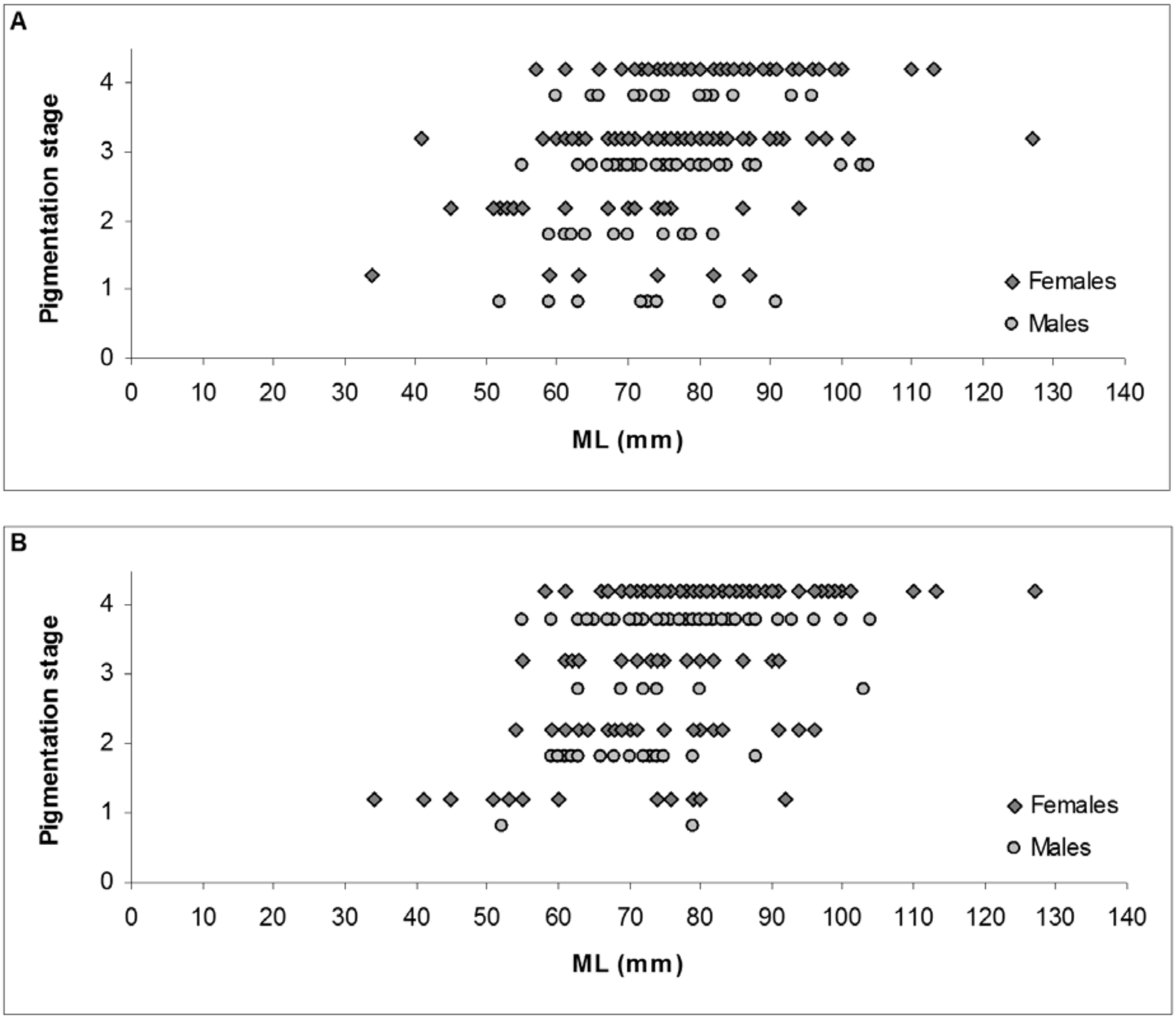

Fig. 3. Mantle length (ML) and pigmentation stages relationship for upper (A) and lower (B) mandible of E. cirrhosa *in Montenegrin waters*

that pigmentation progresses slower compared to the growth in length for (ML), which could be one of the reasons to explain the overlap in the relationship between pigmentation stages and mantle length.

CONCLUSIONS

This study presents the first morphometric analysis of *E. cirrhosa* beaks in the Adriatic Sea. The linear regression analysis has shown higher growth rate of beak characteristics in relation to mantle length and body weight for females with the exception of UCL−ML and LBL−ML relationships, which showed higher growth rate for males. Growth rate of various beak characteristics in relation to hood length also showed higher growth rate for females.

The pigmentation stages were much more easily identified in the lower beak. Darkening of the lateral wall (upper beaks) was shown to progress faster with size (mantle length) for females, while LDLW−LCL and LDLW−ML had higher values for males. There was no clear correlation between beak pigmentation stages and mantle lengths of the *E. cirrhosa*; the pigmentation stages overlapped across most of the recorded mantle length range and therefore pigmentation stages could not be reliably used to estimate the size of an individual.

Of the measured characteristics, the upper mandible lateral wall amplitude and lower mandible wing length showed the highest correlation with mantle length, thus making them the most suitable parameters from which the size of an individual could be most accurately estimated. Similarly, upper mandible crest length represents the optimal choice for the estimation of body weight of *E. cirrhosa.*

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Analiza morfometrije kljuna bijelog muzgavca *Eledone cirrhosa*, Lamarck 1798. (Cephalopoda: Octopoda), u jugoistočnom Jadranu

Zdravko IKICA[1]*, Vladan VUKOVIĆ[1], Mirko ĐUROVIĆ[1], Aleksandar JOKSIMOVIĆ[1] i Svjetlana KRSTULOVIĆ ŠIFNER[2]

[1] *Institut za biologiju mora, Univerzitet Crne Gore, Dobrota b.b., P.P. 69, 85330 Kotor, Crna Gora*

[2] *Sveučilišni odjel za studije mora, Sveučilište u Splitu, Livanjska 5/III, 21000 Split, Hrvatska*

* *Kontakt adresa, e–mail: zdikica@ac.me*

SAŽETAK

Ovaj rad predstavlja prvo istraživanje morfometrije i pigmentacije kljuna bijelog muzgavca, *Eledone cirrhosa* u Jadranskom moru. Analizirano je ukupno 136 kljunova ženki i 82 kljuna mužjaka ove vrste, ulovljenih na području jugoistočnog Jadrana pridnenom povlačnom mrežom – koćom. Omjer mužjaka i ženki bio je 0,6:1. Kljunovi mužjaka su, u odnosu na dužinu plašta, veći od kljunova ženki, ali razlika nije statistički značajna. Rast kljunova u odnosu na dužinu plašta i masu tijela je alometrijski negativan. Slični rezultati zabilježeni su i kod odnosa rasta kljuna prema dužini kape gornjeg i donjeg dijela kljuna, gdje su samo dužina vrška gornjeg dijela kljuna i širina donjeg dijela kljuna kod ženki pokazale vrijednosti parametra *b* veće od 1. Definirana su ukupno četiri stadija pigmentacije gornjeg i donjeg dijela kljuna, s izraženim preklapanjem vrijednosti u odnosu na dužinu plašta.

Ključne riječi: kljun, morfometrija, bijeli muzgavac, *Eledone cirrhosa*, Jadransko more